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305

IRRADIATION OF CELLULAR BLOOD COMPONENTS WITH COBALT 60 IS VERY EFFICIENT AND SAFE IN THE PREVENTION OF TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE (TA-GVHD) IN THE ALLOGENEIC TRANSPLANT SETTING

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For the prevention of TA-GHVH in patients who received a allogeneic stem cell transplant is mandatory the gamma irradiation of the all cellular blood components. This irradiation is usually done with Cesium 137 and with a special blood bank irradiators. However these devices are expensive; because that, in developing countries, is frequent the utilization of Cobalt 60 and the same device that is used in the radiotherapy department, instead of blood bank irradiators. However the information about the efficiency and safety of this procedure is scarce. We present our experience with this technique.

From Dec 2002 to Dec 2005 thirty patients received a allogeneic stem cell transplant and 28 were analysed . The stem cells source was: peripheral blood 25, unrelated cord blood 2, bone marrow 1. The irradiation of the blood components was performed with Cobalt 60 1.24 Mev- (theratron 780 C); the irradiation field was calculated for covering all of the bag surface and a dose of 3.5 Gy was administered to the mild plane of the bag.

158 blood concentrates were transfused, 68 red cell (X:2.5 per patient), and 90 platelets (3.2 per patient). The pre transfusion median hemoglobin and platelet levels were 7.63 g/dl and 12.000/ul; after transfusion was a median increase of 2.3 gm/dl (0.6-4.7) in hemoglobin and 18.000/ul (0-140.000) in platelets.

There was no any case of TA-GVHD. Four patients developed pos transplant aGVHD, in all of the cases the disease began 50 days or more after the last transfusion , there were no pancytopenia and the aGVHD was resolved completely with the treatment.

Conclusion:

In receptors of allogeneic stem cell transplant the gamma irradiation of blood components with Cobalt 60 and the same device wich is used for patients radiotherapy is 100% effective and safe in the prevention of TA-GVHD. This is a very good alternative in centers without a blood bank irradiator.

306

SUCCESSFUL PHASE II TRIAL USING MESENCHYMAL STEM CELLS (MSC) IN COMBINATION WITH STEROID THERAPY FOR THE PRIMARY TREATMENT OF ACUTE GRAFT-VS-HOST DISEASE (AGVHD)

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AGVHD is a major cause of morbidity and mortality after allogeneic stem cell transplantation (SCT). Primary treatment of aGVHD with steroids achieves complete response (CR) rates of only 20-40%. MSC may provide effective GVHD therapy. In this study, Prochymal, an ex-vivo cultured MSC derived from unrelated donors, was used in combination with conventional steroid therapy for primary treatment of aGVHD. Pts were eligible if they had newly diagnosed aGVHD, grades II-IV, after undergoing related or unrelated SCT, or donor lymphocyte infusion (DLI). Study endpoints were drug safety and aGVHD response rates by day 28 after infusion. Pts were randomized to 2 doses of Prochymal: 2 (low) or 8 million (high) cells/kg. Prochymal was initiated along with steroid therapy at time of aGVHD diagnosis. 2 Prochymal infusions were administered 3-5 days apart within 72 hrs of steroid initiation. Tacrolimus, cyclosporine, or MMF were maintained for GVHD prophylaxis . Pts were maintained on steroids (2 mg/kg/d) for at least 1 week with objective of subsequently tapering off steroids. 32 pts (23 males, 9 females) were enrolled, and 31 were evaluable with median age 52 yrs (range 34-67). AGVHD developed following matched sibling (n=15) or matched unrelated SCT (n=13), or DLI (n=4). Distribution of aGVHD is described in table below. Pts were randomized to low (n=17) or high (n=15) Prochymal dose. 90% of pts (n=28) initially responded to aGVHD treatment: 21 achieved CR with no evidence of GVHD, and 7 achieved PR with a reduction in 1 organ stage. 100% of pts initially diagnosed with skin GVHD had a response to treatment, and 83% of pts with GVHD involving GI alone or with other organs had a response. 9 pts (31%) eventually required a second line agent to control aGVHD. Non-relapse survival at day 100 was 79.3% with 8 pts dying at a median of 48 days (range 13-58): aGVHD (n=2), intracranial bleed (n=1), relapse (n=1), infection (n=4). No infusional toxicities or discontinuation of treatment was observed. 1 pt developed atrial fibrillation 24 hrs following the second Prochymal infusion. No ectopic tissue formation was noted by CT scans at day 28. Addition of Prochymal to standard steroid therapy for the primary treatment of aGVHD resulted in a high response rate with minimal added toxicity. This trial demonstrates the potential of using a universal, cellular product for the treatment of aGVHD. A phase III clinical trial has been initiated to confirm these promising results.

Grade and Distribution of GVHD

Grade (no. pts)	**II(21)**	**III (8)**	**IV(3)**	
Organ (no. pts)	**GI(12)**	**Skin(13)**	**GI/Skin(5)**	**GI/Liver(2)**

307

INITIAL SELECTION OF HIGH AFFINITY CD25+ CELLS INCREASES THE PURITY OF CD4+CD25+FOXP3+ T REGULATORY CELLS EXPANDED IN MEDIUM CONTAINING RAPAMYCIN

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CD4+ T regulatory cells (Tregs) with potent suppressor activity are marked by high levels of CD25 and expression of the transcription factor, Foxp3. We described an approach to isolate and expand Tregs using a single CD25-enrichment on a MACS column, activation by CD3/CD28-coated beads, and culture in medium containing rapamycin (Keever-Taylor et al, submitted). We achieved 10-fold expansion at day 10 of cells with potent suppressor activity that were enriched for CD4+Foxp3+CD27+ cells using 1-10 ng/mL rapamycin. After 21 days the cells expanded 200-fold but although still enriched for CD4+Foxp3+CD27+ cells compared cultures grown without rapamycin, the suppressor activity and Foxp3 content had greatly declined. We have further optimized conditions by exploring two approaches: 1) increase the starting purity of CD4+Foxp3+ cells, and 2) increase the dose of rapamycin to further inhibit the expansion of activated T cells. We compared the purity of Tregs when 1, 2, or the previously used 4 μL of CD25-coated beads/10^7 starting cells were used to bind the CD25+ cells and 1 versus 2 selection columns. Data showed that after passage through one column the % of CD4+ cells expressing Foxp3 was 27.3%, 54.3% and 31.7% with 1, 2, or 4 μL beads, respectively. The %CD4+Foxp3+ cells further increased to 51.9%, 64.1% and 60%, respectively after a passage through a second column. Therefore, subsequent experiments used 2 μL beads and sequential passage through two columns. Doses of rapamycin from 50-200 ng/mL were increasing toxic, with few cells recovered from the expansion cultures. However, at the previously used doses of 5-10 ng/mL the %CD4+Foxp3+ cells after 22-24 days averaged 37.3±7.1%, similar to the 42±20% seen at day 10 in our previous experiments and significantly higher than the 23±2% previously seen at day 21 when suppressor activity had declined. In contrast only 4.7±0.4% of the recovered CD4+ cells expressed Foxp3 in the absence of rapamycin, similar to the 5±3% shown for single

column-passed fractions at day 21. In summary our data show that by increasing the starting purity of CD4+Foxp3+CD27+ cells a greater expansion of the Treg population can be achieved, making it more feasible to obtain clinically useful doses of Treg for immunotherapy protocols to prevent or treat GVHD. Rapamycin at doses of 5-10 ng/mL inhibits the expansion of cells without the phenotype and function of Tregs, but higher doses (50-200 ng/mL) are toxic to Tregs.

308

TREATMENT OF STEROID REFRACTORY, SEVERE ACUTE GRAFT VERSUS HOST DISEASE WITH EXPANDED MESENCHYMAL STEM CELLS IN CHILDREN HAVING UNDERGONE ALLOGENEIC STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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Despite advances in pre-transplant immune suppression and donor HLA typing methods acute graft versus host disease (aGvHD) remains a significant problem following allogeneic HSCT. Steroid therapy is the treatment of choice in severe aGvHD. Steroid non-responsive aGvHD is associated with increased morbidity and more importantly death due to organ damage and/or infection related to the use of continuing immune suppression. Second line treatments continue to be evaluated. Whatever their initial effects, presently they have had little impact on overall survival.

Mesenchymal stem cells (MSCs) are poor antigen presenting cells, not expressing MHC class II or co-stimulatory molecules. They down regulate allo-reactive T cell responses when added to mixed lymphocyte cultures. MSCs alter cytokine excretion profiles of dendritic cells, naïve and effector T cells, and NK cells inducing a more tolerant phenotype.

MSCs have been used successfully in a child with resistant aGvHD (le Blanc. Lancet 2004).

We conducted an ethically committee approved prospective phase I/II study of co-infusion of expanded MSCs for treatment of children with steroid refractory, grade II-IV aGVHD.

MSCs, isolated from parental donor marrow were expanded under GMP conditions. MSCs either as haploidentical or 3rd party.

Patient characteristics receiving MSCs for steroid refractory GvHD. LUMC 2005-2006

UPN 1	UPN 2	UPN 3	UPN 4
Male	Male	Male	Female
MDS RC	Omens	Fanconi anemia	JMML
12y, 3mo	1 yr, 4mo	6yr, 10mo	1yr, 7mo
Sibling ID	ORD ID x 2	Matched UD BMT	Matched Cord Blood
GvHD 4 (Skin/GI/ Liver)	4 GI acute/chronic plus CMV/graft failure	4 (skin/GI/ Liver)	4 GI plus AdV
Steroid/CSA/ Tacrolimus/ MMF	Steroid/ Tacrolimus/ MMF	Steroid/CSA/ Anti TNFα/ anti CD25	Steroid/ Tacrolimus/ MMF
MSC1 3rd party	MSC2 haplo	MSC1 3rd party	MSC2 3rd party
1.1 × 106/kg	1.8 × 106/kg	2.3 × 106/kg	1.76 × 106/kg
Male 33 years	Female 25 years	Male 33 years	Female 25 years
2 infusions	2 infusions	2 infusions	2 infusions
4→0 CR	No response	4→0 CR	4→0 CR
Died Klebsilella sepsis	Died CMV/GvHD	Alive Limited cGvHD skin	Alive small bowel fibrosis
UPN 5	UPN 6	UPN 7	UPN 8
Male	Female	Male	Female
Kostmaan syndrome	EBV induced HLH	MDS RC	MDS RC monosomy 7
3yr, 10mo	6yr, 4mo	13yr, 2 mo	4yr, 2mo
Matched UD BMT	Cord Plus Haplo and MM UD BMT	MUD x 3, DLI's	MM Cord blood
4GI acute/ chronic	4 Liver	4 (Skin/GI/ Liver)	4 (Skin/GI/Liver) AdV
Steroid/CSA/ Anti TNFα/ anti CD25	Steroid/CSA/ Tacrolimus	Steroid/MMF	Steroid/CSA/ Tacrolimus
MSC3 3rd party	MSC2 3rd party	MSC1 3rd party	MSC4 3rd party
1.7 ×106/kg	2.0 ×106/kg	1.2 × 106/kg	2.2 × 106/kg
Female 35 years	Female 25 years	Male 33 years	Male 36 years
2 infusions	1 infusion	2 infusions	2 infusions
PR; relapsed GvHD	Not evaluated	4→0 CR	4→0 CR
Died infection/ GvHD	Died EBV reactivation	Alive liver dysfunction	Alive

UPN - Unique patient number; CR - complete response: PR - partial response; AdV - adenovirus; CMV - cytomegalovirus; EBV - Epstein Barr virus; HLH - hemaphagocytic lymphohistiocytosis; MDS RC - myelodysplastic syndrome and refractory cytopenia; JMML - Juvenile myelomonocytic leukemia; CSA - cyclosporine

309

BLOCKING LFA-1 ACTIVATION WITH LOVASTATIN PREVENTS GRAFT-VERSUS-HOST DISEASE IN MOUSE BONE MARROW TRANSPLANTATION

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Leukocyte function associated antigen-1 (LFA-1) regulates T cell adhesion and activation. LFA-1 is constitutively expressed on cell surface in an inactive state. The control of LFA-1 activation is critical in inflammatory and immune responses. We demonstrated previously that the I-domain, the ligand binding site of LFA-1, changes from the low-affinity state to high-affinity state upon activation. Therapeutic antagonist, such as lovastatin, stabilizes the I-domain in the low-affinity state and inhibits the LFA-1 activation. Here, we report that lovastatin can block mouse T cell adhesion and proliferation in vitro. First, we demonstrated that lovastatin treatment reduced the mortality and morbidity in the mouse GVHD model. Lovastatin treatment significantly decreased GVHD mortality with 80% mice survived over 28 days, whereas more than 70% of the control mice died within the first 10 days, and the p values was 0.045. There were significantly reduced tissue damages in the skin, intestine and liver of lovastatin-treated mice. Second, we found lovastatin treatment reduced donor T cell homing to lymph nodes. There was a 65% reduction of CD4+ T cells homing to lymph nodes in lovastatin treatment group compared to control. The reduction of CD8+ T cells was greater with about 76% less cells homing to lymph nodes in the lovastatin treatment group. Third, we found lovastatin treatment reduced donor-derived T cell proliferation in vivo. There were 37% CD4+ and 31% CD8+ T cells remained undivided in the lymph nodes of the control mice at day 4 post-transplant. The lovastatin-treated mice had reduced proliferation kinetics of both CD4+ and CD8+ T cells with about 55% and 42% remained undivided. In the control lymph nodes, there were 42% CD4+ and 59% CD8+ T cells